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14. ABSTRACT:

CML results from a chromosomal translocation in hematopoietic stem cells (HSC), yet the disease primarily presents as a myeloid hyperplasia with relatively infrequent lymphoid involvement. We proposed that age-associated defects in the potential of HSC to generate lymphocytes underlies this presentation. The results at the time of this writing support this hypothesis. Bone marrow cells from young and old mice were transduced with a retrovirus carrying the BCR-ABL 9:22 chromosomal translocation and then transplanted into young recipients. The data indicate that recipients of the young,transduced bone marrow cells presented with myeloid and lymphoid leukemias. In contrast, recipients of old, transduced bone marrow developed leukemia with infrequent lymphoid involvement. Ongoing studies are aimed at identifying the leukemia stem cells in the young and old bone marrow.

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INTRODUCTION

Chronic myeloid leukemia (CML) is a clonal hematopoietic malignancy characterized by myeloid hyperplasia. CML results from expression of the t(9;22) chromosomal translocation between the BCR and ABL genes and encodes a protein that mediates altered kinase activity in hematopoietic precursors Patients typically present with an increased number of mature neutrophils, basophils and eosinophils and their precursors in peripheral blood (1-9), and this increased white blood cell count is accompanied by a marked myeloid hyperplasia in the bone marrow (BM; 3,6). This initial, chronic phase of disease is followed by blast crisis in which immature progenitors predominate (7). Although B lineage blasts are observed in some patients, T cell blast crisis occurs infrequently and this blast crisis phase of disease is also myeloid dominant (1,3,6,7). The aim of the studies proposed that have been conducted are to:

i. determine if expression of the BCR-ABL gene is relatively inefficient in lymphoid progenitors or whether lymphoid progenitors in which BCR-ABL is expressed have a growth disadvantage.

ii. determine if the infrequenct lymphoid involvement in CML is due to age-associated defects in lymphopoiesis that in turn result in fewer lymphoid target cells.

In order to address these issues, bone marrow cells from young and old mice were transduced with a retrovirus carrying BCR-ABL and then transplanted into young recipients. Whether or not the mice developed leukemia was determined, and if so, whether the presentation was myeloid or lymphoid was assessed by a lineage analysis of blood cell populations in their bone marrow, thymus, spleen, and lymph nodes.

BODY

CML presents primarily as a myeloid hyperplasia in patients in both its acute and chronic phases. When malignant lymphoid cells are observed, they are primarily B lymphoid lineage in origin. This overwhelming presentation of myeloid cell dominance in CML is puzzling given that the chromosomal translocation in CML occurs in hematopoietic stem cells (HSC).

In view of these points, the objectives of this proposal are to address the following two questions:

1. Are myeloid progenitors more susceptible to the effects of BCR-ABL than lymphoid progenitors?

2. How does aging affect CML progression?

These questions were addressed by performing the following tasks:

Task 1: Enrich lineage negative (Lin-) c-kit^{hi} Sca-1^{hi} HSC, Lin- Sca-1- c-kit+ CD127 (IL-7Rα)- Common Myeloid Progenitors (CMP) and Lin- Sca-1^{lo} c-kit^{lo} CD127 (IL-7Rα)+ Common Lymphoid Progenitors (CLP) from young and old BALB/c mice;

Our laboratory has developed the procedures to label bone marrow cells with antibodies to multiple cell surface determinants and to isolate various hematopoietic progenitor populations by flow cytometry. We now have considerable expertise in the isolation of HSC, common lymphoid progenitors (CLP), common myeloid progenitors (CMP), and committed lymphoid and myeloid progenitors. Evidence documenting this expertise can be found in a recent paper from the laboratory published in the *Journal of Immunology* (10).

Task 2: Culture cells in medium, cytokine cocktail (IL-3, IL-6, c-kit ligand for HSC and CMP and IL-3, IL-6, c-kit ligand, IL-7 for CLP), and retroviral vector containing either 5' long terminal repeat (LTR)-driven BCR-ABL^{P210} internal ribosome entry site (IRES) enhanced green fluorescent protein (EGFP) or 5' LTR-driven IRES EGFP for 24-36 hours;

We received the BCR-ABL construct from our collaborator, Dr. Owen Witte. This gene was then inserted into a murine retrovirus. We then prepared stocks of retrovirus to be used for transduction of hematopoietic cells. A particular issue with some transduction protocols is that they involve the culture of purified hematopoietic stem cells with retrovirus for several days, and in our original application we indicated that our incubation time would be between 24-36 hours.. However, a problem with this approach is that the stem cells may have differentiated by this time. Therefore, we developed a methodology to incubate bone marrow cells with retrovirus plus cytokines for only six hours.

Task 3: Inject low (10^5) and high (10^6) doses of transduced cells into groups of six lethally irradiated, syngeneic recipients;

We have now repopulated several groups of mice with BCR-ABL transduced bone marrow cells from young and old mice. These mice have developed leukemia, and they were analyzed as described below in Task 5.

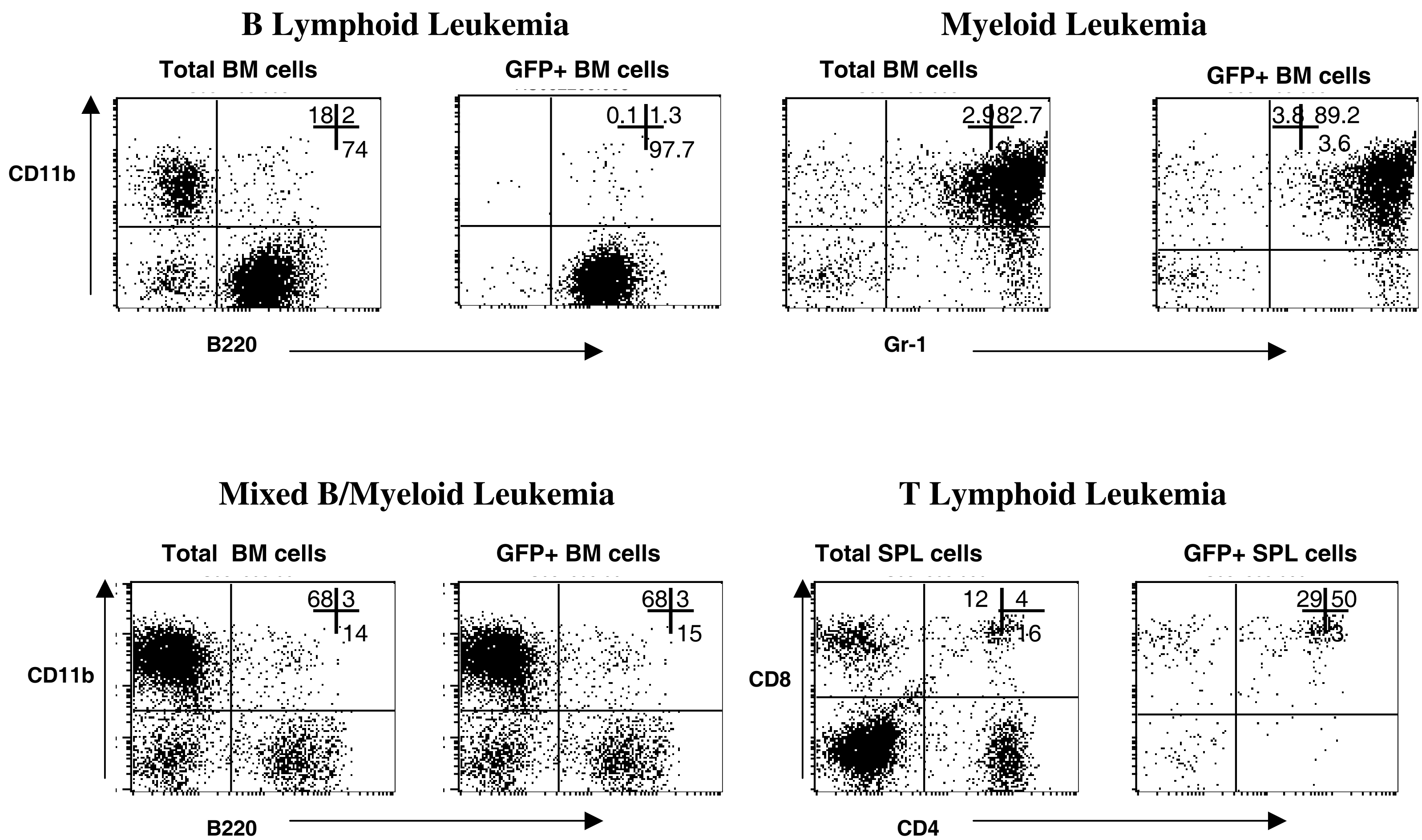
Task 4: Analyze peripheral blood at 2 weeks post-transplantation for morphology and cell phenotype;

The submitted application indicated that we would analyze peripheral blood of mice two weeks following the transplantation of BCR-ABL infected bone marrow into them. However, we have found that mice reliably develop leukemia at 4-8 weeks following transplantation. Therefore, we have not performed the peripheral blood analysis at earlier time points, because this is a stressful procedure requiring that mice be anesthetized and bled via the retro-orbital sinus.

Task 5: Sacrifice mice when animals appear diseased, perform histopathologic and phenotypic analysis on GFP+ (donor derived) and GFP- (endogenous host) bone marrow, spleen, lymph nodes, thymus, and peripheral blood cells in all the animals. In addition, confirm integration of BCR-ABL by PCR and Southern blotting;

We have now processed 20 mice that were repopulated with young, BCR-ABL transduced bone marrow and 15 mice that received a transplant of old BCR-ABL infected bone marrow cells. Because the retrovirus in which the BCR-ABL gene was inserted is bi-cistronic and contains the gene encoding green fluorescence protein (GFP) in addition to BCR-ABL, we were able to assess the repopulation in the myeloid ($CD11b^+$ and $Gr\text{-}1^+$), B lymphoid ($B220^+$), and T ($CD4^+$ and $CD8^+$) lineages. An example of how this analysis is performed is shown in Figure 1.

Leukemia Classification

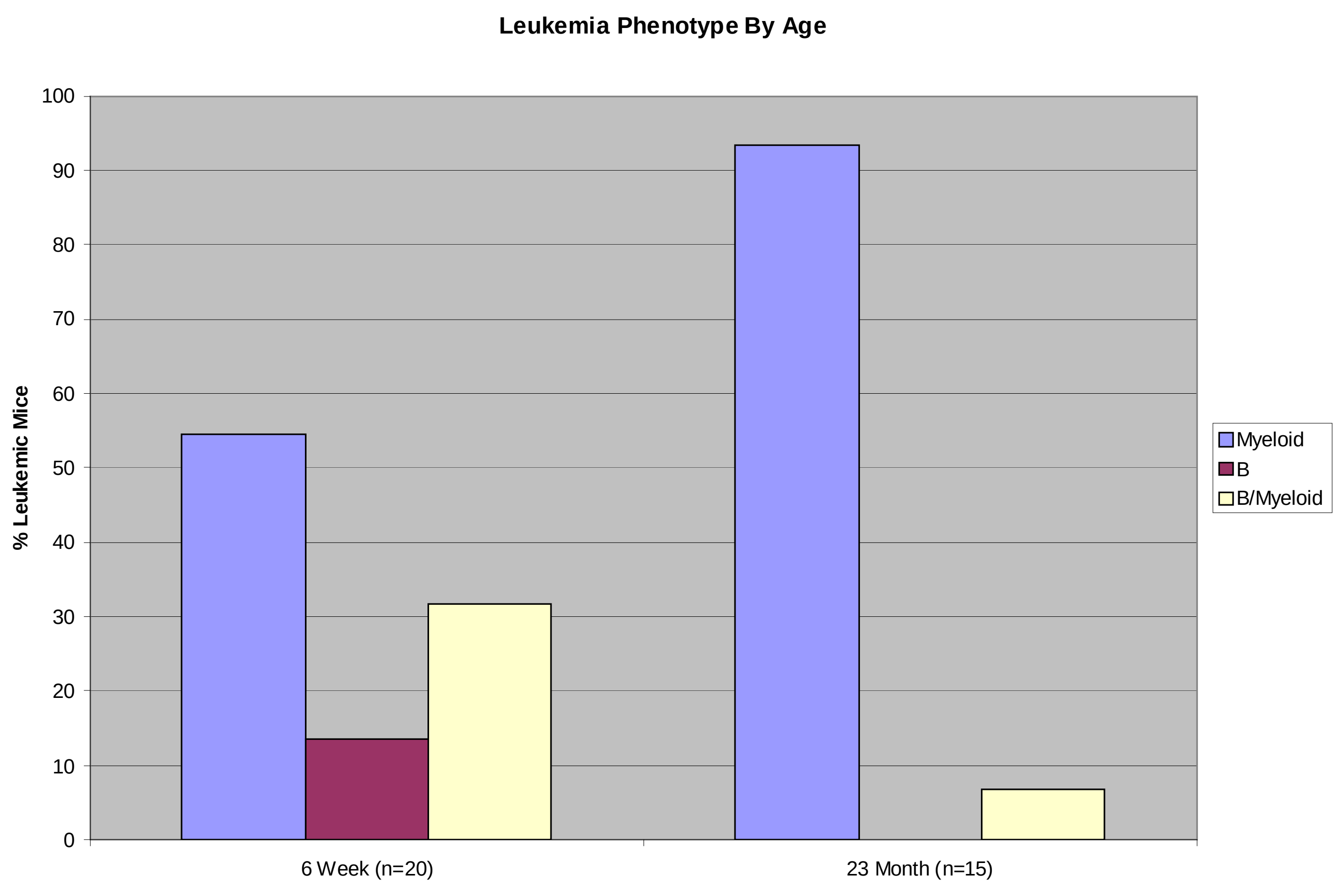


Task 6: Analyze data and determine how the lineage and age of the progenitor influences disease progression.

We have analyzed the 25 mice that received young, BCR-ABL transduced cells and the 15 mice that received old transduced cells in the manner described above, and Figure 2 presents a summary of these results.

The data are striking in that the mice that received young cells developed leukemias that were both myeloid and lymphoid in their presentation. In contrast, mice that received old cells developed leukemias, but these were almost exclusively myeloid in their presentation.

These data are being prepared for publication. To our knowledge, this will be the first report to link changes in lymphopoiesis with aging as a factor that explains the presentation of CML.



KEY RESEARCH ACCOMPLISHMENTS

- Developed methodologies to purifiy hematopoietic progenitor populations and to infect them with retroviral vectors containing the BCR-ABL gene
- Demonstrated that the pattern of BCR-ABL mediated leukemia is influenced by the age of the hematopoietic targets

REPORTABLE OUTCOMES

- An abstract describing this work will be presented at the 4th International Society for Stem Cell Research meeting in Toronto in June 2006. We are awaiting notification as to whether the format will be a poster or oral presentation.

- A manuscript describing this work will be prepared in Spring/Summer 2006

- The award supported the doctoral thesis work of Robert Signer, a graduate student enrolled in the Cellular and Molecular Pathology Graduate Program at UCLA.

CONCLUSION

The importance of this work is that it provides an understanding of why CML presents clinically as a predominantly myeloid disease. Specifically, the minor lymphoid involvement reflects the fact that the lymphoid developmental potential of aged hematopoietic stem and progenitor cell populations is severely compromised.

These results suggest a next step, which is to identify specific reasons why lymphoid developmental potential of aged hematopoietic cells is compromised. We will be extending our work to examine the role that epigenetic changes play in this phenomenon.

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APPEDICES

None

SUPPORTING DATA

None